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Middle East Respiratory Syndrome Coronavirus Infection Not Found in Camels in Japan

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Middle East Respiratory Syndrome (MERS) is an emerging respiratory disease caused by a newly identified coronavirus the MERS coronavirus (MERS-CoV) (1–3). The outbreak has mainly remained in Saudi Arabia since 2012, with 1,106 laboratory confirmed cases to date, which have resulted in 421 deaths as of April 16, 2015 (The World Health Organization [WHO], Global Alert and Response [GAR], Coronavirus infections, updated on April 16, 2015, http://www.who.int/csr/don/16-april-2015-mers-saudi-arabia/en/).

Initially, bats were considered the main reservoir (4). However, recent reports suggest that dromedary camels are the most likely candidates for the natural reservoir of MERS-CoV, as a form of the virus has been circulating in camels in Saudi Arabia since at least 1992 (5–8). In addition, an experimental infection into dromedary camels resulted in successful infection with mild respiratory symptoms (9). These observations suggest that epidemiological surveillance of dromedary camels for MERS-CoV is rather important to understand the biorisk of MERS-CoV infection from dromedaries inside the country. Therefore, in the present study, dromedary camels living in Japan were tested for MERS-CoV.

Specimens from dromedary camels were collected by collaboration with the Japanese Association of Zoos and Aquariums, Tokyo, Japan. All specimens were collected in compliance with the ethical policies at each institution. A total of 18 fecal specimens, 10 saliva specimens, 4 nasal swabs, and 5 serum specimens were obtained from 20 dromedaries, which is representative of almost 87% of the dromedaries in Japan. As most dromedaries in Japan are not trained for riding, it becomes necessary to anesthetize the untrained animals prior to blood collection for the safety of the staff involved; however, anesthesia poses a high-risk for aged camels. Therefore, serum specimens were only collected from 2 dead camels and 3 camels trained for riding. Feces were collected from all 20 animals and stored at −80°C until further uses. Saliva and nasal swabs were collected using UTM Virus Collection (360C, Copan Diagnosis, Brescia, Italy) depending on the breeding condition. Detection of MERS-CoV was performed by 2 MERS-CoV-specific assays–real-time RT-PCR (upE and ORF1 a probe sets) (10,11) and reverse transcription-loop-mediated isothermal amplification (RT-LAMP) (12) and a comprehensive assay using conventional RT-PCR using a primer set for pan-coronaviruses (13). Viral RNAs were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For fecal specimens, before RNA isolation, 10% homogenates in 10% chloroform-PBS were prepared, which were vortexed for 30 min at room temperature and then centrifuged. The supernatants were used for RNA isolation. The protocols for RT-PCR were previously described (10–13). Five microliters of RNA were used for each amplification reaction. To detect antibodies for MERS-CoV in serum, a neutralizing assay was performed using Vero/TMPRSS2 cells constitutively expressing type II transmembrane serine protease (TMPRSS2) (14), which enhances cell entry and fusion formation of MERS-CoV (15,16). Then, 50 plaque forming unit of MERS-CoV (EMC isolate) were mixed with serially diluted camel serum and incubated at 37°C for 45 min, after which it was inoculated onto Vero/TMPRSS2 cells formed in a 96-well plate. After virus adsorption, the cells were washed and incubated at 37°C in Dulbecco's Modified Eagle's Medium containing 5% fetal calf serum. After a 24-h incubation, the number of syncytia formed was calculated. Mouse monoclonal antibodies immunized with UV-inactivated MERS-CoV were used as a positive control for neutralization. A specimen that showed 80% neutralization of more than 20 × dilution was considered positive for MERS-CoV infection.

The list of specimens and results is shown in Tables 1 (genetic diagnostic methods) and 2 (neutralizing assay).

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Table 1. The list of specimens and results of MERS-CoV RNA detection from dromedaries

Zoo	Age	Sex	Specimen	TaqMan assay				Comment
				upE	ORF1a	RT-LAMP	RT-PCR	
A	22	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	19	Male	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
B	4	Female	Feces	Neg	Neg	Neg	Neg	Trained for riding
			Saliva	Neg	Neg	Neg	Neg	
			Nasal swab	Neg	Neg	Neg	Neg	
C	20	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
			Nasal swab	Neg	Neg	Neg	Neg	
D	19	Female	Feces	Neg	Neg	Neg	Neg	
	8	Male	Feces	Neg	Neg	Neg	Neg	
E	1	Male	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	14	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	2	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	18	Male	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	1	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
	3	Female	Feces	Neg	Neg	Neg	Neg	
			Saliva	Neg	Neg	Neg	Neg	
F	15	Male	Feces	Neg	Neg	Neg	Neg	
	29	Female	Feces	Neg	Neg	Neg	Neg	
	20	Female	Feces	Neg	Neg	Neg	Neg	
	17	Female	Feces	Neg	Neg	Neg	Neg	
G	10	Male	Feces	Neg	Neg	Neg	Neg	Trained for riding
			Nasal swab	Neg	Neg	Neg	Neg	
	9	Female	Feces	Neg	Neg	Neg	Neg	Trained for riding
			Nasal swab	Neg	Neg	Neg	Neg	

Neg: negative for MERS-CoV RNA.
Zoo name: A, Kujukushima Zoological and Botanical Garden; B, Zoorasia Yokohama Zoological Gardens; C, Hirakawa Zoological Park; D, Himeji City Zoo; E, Gunma Safari Park; F, Adventure World; G, Rakudaya.

Table 2. Neutralizing assay for MERS-CoV using serum specimens from dromedaries

Zoo	Age	Sex	Antibody titer	Comment
B	4	Female	<20	Trained for riding
E	0	Female	<20	Died of heatstroke
F	26	Female	<20	Died from natural causes
G	10	Male	<20	Trained for riding
	9	Female	<20	Trained for riding

See footnote of Table 1.

All the specimens were negative for viral RNA by the MERS-CoV specific assays (TaqMan and RT-LAMP). In 2 specimens, RT-PCR with pan-coronavirus primers showed non specific bands; however, sequencing analysis of the amplicons showed no coronaviral sequences. The neutralizing antibody titers in serum specimens for MERS-CoV were also less than 20 × dilution, and hence considered negative for MERS-CoV infection.

The other species of camels besides dromedaries are Bactrian camels, and wild Bactrian camels are distributed around Central Asia. Although a hybrid of dromedaries and Bactrian camels can be bred, there is no obvious evidence of the MERS-CoV infection in Bactrian camels. Reusken et al. (17) investigated 2 Dutch Bactrian camels that showed seropositivity for human coronavirus OC43, but not for MERS-CoV. However, epidemiological surveillance of MERS-CoV infection in Bactrian camels has been very limited. In zoo G, 6 Bactrian camels have been bred (2 imported from The Netherlands) and are used for camel riding, and these camels have frequent contact with humans. Therefore, these Bactrian camels were tested for MERS-CoV by genetic and serological methods. As shown in Table 3, all 6 camels were negative for MERS-CoV. Collectively, these results suggest that dromedary and Bactrian camels in Japan are free from MERS-CoV infection.

The rate of positive antibodies in dromedary camels is quite high in the Middle East and North African countries (17). However, the rate decreases farther from these regions: it is about 30%–50% in Tunisia and about 13% in the Canary Islands (17). It has also been reported that dromedaries in Australia are free of the MERS-CoV infection (18). Therefore, these findings indicate that the MERS-CoV infection is location specific

Table 3. MERS-CoV tests for the specimens from Bactrian camels

Zoo	Age	Sex	Specimen	TaqMan assay				Neutralizing assay	Comment
				upE	ORF1a	RT-LAMP	RT-PCR		
G	3	Female	Feces	Neg	Neg	Neg	Neg		Imported from The Netherlands in 2013
			Nasal swab	Neg	Neg	Neg	Neg		Trained for riding
			Serum					<20	
	2	Female	Feces	Neg	Neg	Neg	Neg		Imported from The Netherlands in 2013
			Nasal swab	Neg	Neg	Neg	Neg		Trained for riding
			Serum					<20	
	0	Male	Feces	Neg	Neg	Neg	Neg		
	18	Female	Feces	Neg	Neg	Neg	Neg		Trained for riding
			Nasal swab	Neg	Neg	Neg	Neg		
			Serum					<20	
	16	Female	Feces	Neg	Neg	Neg	Neg		Trained for riding
			Nasal swab	Neg	Neg	Neg	Neg		
			Serum					<20	
	13	Female	Feces	Neg	Neg	Neg	Neg		Trained for riding
			Nasal swab	Neg	Neg	Neg	Neg		
			Serum					<20	

See footnote of Table 1.

and does not infect all dromedaries worldwide. In Japan, all dromedary camels are only bred inside the country; there have been no reported importations of dromedary camels from MERS-CoV endemic areas for several decades. In contrast, the 2 Bactrian camels tested in this study were imported from The Netherlands. However, there have been no reports that Bactrian camels are positive for MERS-CoV. Collectively, these findings suggest that the possibility of MERS-CoV infection in camels in Japan is extremely low. However, for public health purposes, it is important to continue the epidemiological surveillance of camels for MERS-CoV infection.

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Conflict of interest None to declare.

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